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(54) Title: ANGIOPOIETIN–LIKE PROTEINS, CRISP PROTEINS AND POLYNUCLEOTIDES ENCODING SAME

(57) Abstract

Angiopoietin–like protein 2, angiopoietin–like protein 3, Apple Crisp protein and Cocoa Crisp protein are disclosed. Polynucleotides encoding such proteins are also provided.

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ANGIOPOIETIN-LIKE PROTEINS. CRISP PROTEINS AND POLYNUCLEOTIDES ENCODING SAME

BACKGROUND OF THE INVENTION

Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (i.e., partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these proteins and the polynucleotides encoding them that the present invention is directed.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 433 to nucleotide 1905;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR2 deposited under accession number ATCC 207060;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR2 deposited under accession number ATCC 207060;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:9;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:9 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:8.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:9;

(b) fragments of the amino acid sequence of SEQ ID NO:9; and

(d) the amino acid sequence encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

the protein being substantially free from other mammalian proteins.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 358 to nucleotide 1836;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR3 deposited under accession number ATCC 207063:

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR3 deposited under accession number ATCC 207063;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above:

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:13.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:14;

(b) fragments of the amino acid sequence of SEQ ID NO:14; and

(d) the amino acid sequence encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

the protein being substantially free from other mammalian proteins.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:20;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:20 from nucleotide 50 to nucleotide 775;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone APPLE deposited under accession number ATCC 207061;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone APPLE deposited under accession number ATCC 207061;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:21;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:21 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:20.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:21;

(b) fragments of the amino acid sequence of SEQ ID NO:21; and

(d) the amino acid sequence encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

the protein being substantially free from other mammalian proteins.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:23;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:23 from nucleotide 65 to nucleotide 1564;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone COCO deposited under accession number ATCC 207062;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone COCO deposited under accession number ATCC 207062;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062:

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:24;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:24 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:23.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:24;

(b) fragments of the amino acid sequence of SEQ ID NO:24; and

(d) the amino acid sequence encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062;

the protein being substantially free from other mammalian proteins.

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions. Also provided by the present invention are organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein.

Processes are also provided for producing a protein, which comprise:

(a) growing a culture of the host cell transformed with such polynucleotide compositions in a suitable culture medium; and

(b) purifying the protein from the culture.

The protein produced according to such methods is also provided by the present invention. Preferred embodiments include those in which the protein produced by such process is a mature form of the protein.

Protein compositions of the present invention may further comprise a pharmaceutically acceptable carrier. Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and a pharmaceutically acceptable carrier.

Antibodies directed to the proteins disclosed herein are also provided as well as methods for using such antibodies for therapeutic treatment.

DETAILED DESCRIPTION

ISOLATED PROTEINS AND POLYNUCLEOTIDES

As used herein a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g. , receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins which are transported across the membrane of the endoplasmic reticulum.

Angiopoietin-Like Protein 2 (AR2) and Angiopoietin-Like Protein 3 (AR3)

A yeast signal sequence trap similar to that described in U.S. Patent No. 5,536,637 was used to identify secreted ESTs (sESTs) from a chicken embryonic limb bud library. An sEST was identified and used to clone a corresponding full-length chicken cDNA, GW75 (SEQ ID NO:1, SEQ ID NO:2).

Using the chicken GW75 full-length sequence to screen the public EST database, two partial human homolog sequences were identified. GenBank AA463383 is reported at SEQ ID NO:3 and GenBank T11442 is reported at SEQ ID NO:4. Oligonucleotides were designed to these ESTs and partial full-length human clones were isolated. A second set of oligonucleotides to the partial clones were then used to isolate the full-length human cDNAs.

The full-length cDNAs were isolated from a dT-primed cDNA library constructed in the plasmid vector pED-dpc2. cDNA was made from human fetal heart RNA purchased from ABR.

The probe sequences used to isolate AR2 were derived from EST fragment AA463383 (SEQ ID NO:3). The sequence of the two probes are provide as SEQ ID NO:5 and SEQ ID NO:6. The probes were antisense to the EST.

The probe sequences used to isolate AR3 were derived from EST fragment T11442 (SEQ ID NO:4). The sequence of the two probes are provide as SEQ ID NO:10 and SEQ ID NO:11. The probes were also antisense to the EST.

The DNA probes were radioactively labeled with 32P and used to screen the human fetal heart dT-primed cDNA library, under high stringency hybridization/washing conditions, to identify clones containing sequences of the AR2 and AR3 genes.

One hundred thousand library transformants were plated at a density of approximately 5000 transformants per plate on separate sets of 20 plates each. Nitrocellulose replicas of the transformed colonies were hybridized to the 32P labeled DNA probes in standard hybridization buffer (6X SSC, 0.2% nonfat dried milk, 0.0008% sodium azide, 1% NP-40 and 0.05% sodium pyrophosphate) under high stringency conditions (65°C for 2 hours). After 2 hours hybridization, the radioactively labeled DNA probe containing hybridization solutions wre removed and the filters were washed under high stringency conditions (3X SSC, 0.05% sodium pyrophosphate, 21°C for 5 minutes standing; followed by 2.2X SSC, 0.05% sodium pyrophosphate, 21°C for 15 minutes shaking; followed by a second 2.2X SSC, 0.05% sodium pyrophosphate, 65°C for 1 minute shaking). The filters were wrapped in Saran wrap and exposed to x-ray film overnight to 3 days at –80°C. The autoradiographs were developed and positively hybridizing transformants of various signal intensities were identified.

These positive clones were picked, grown for 5 hours in selective medium and plated at low density (approximately 300 colonies per plate). Nitrocellulose replicas of the colonies were hybridized to the 32P labeled probes in standard hybridization buffer (6X SSC, 0.2% nonfat dried milk, 0.0008% sodium azide, 1% NP-40 and 0.05% sodium pyrophosphate) under high stringency conditions (65°C for 2 hours). After 2 hours hybridization, the radioactively labeled DNA probe containing hybridization solutions were removed and the filters were washed under high stringency conditions (3X SSC, 0.05% sodium pyrophosphate, 21°C for 5 minutes standing; followed by 2.2X SSC, 0.05% sodium pyrophosphate, 21°C for 15 minutes shaking; followed by a second 2.2X SSC, 0.05% sodium pyrophosphate, 65°C for 1 minute shaking). The filters were wrapped in Saran wrap and exposed to x-ray film overnight to 3 days at –80°C. The autoradiographs were developed and positively hybridizing transformants were identified.

Bacterial stocks of purified hybridization positive clones were made and plasmid DNA was isolated. The sequence of the cDNA insert of each transformant was determined.

In both instances, the cDNA insert contained sequences of both probes used in the hybridization. However, also in both instances, the isolated clones did not appear to have a complete open reading frame as compared to other family members. As a result, a third probe was designed which was antisense to each partial clone. The sequence of the probe designed from the partial clone corresponding to AA463383 is reported as SEQ ID NO:7. The sequence of the probe designed from the partial clone corresponding to T11442 is reported as SEQ ID NO:12.

Eighty thousand library transformants were plated at a density of approximately 4000 transformants per plate on 20 plates for AA4653383. One hundred thirty thousand library transformants were plated at a density of approximately 6500 transformants per plate on 20 plates for T11442. Nitrocellulose replicas of the colonies were hybridized to the 32P labeled probes in standard hybridization buffer (6X SSC, 0.2% nonfat dried milk, 0.0008% sodium azide, 1% NP-40 and 0.05% sodium pyrophosphate) under high stringency conditions (65°C for 2 hours). After 2 hours hybridization, the radioactively labeled DNA probe containing hybridization solutions were removed and the filters were washed under high stringency conditions (3X SSC, 0.05% sodium pyrophosphate, 21°C for 5 minutes standing; followed by 2.2X SSC, 0.05% sodium pyrophosphate, 21°C for 15 minutes shaking; followed by a second 2.2X SSC, 0.05% sodium pyrophosphate, 65°C for 1 minute shaking). The filters were wrapped in Saran wrap and exposed to x-ray film overnight to 3 days at –80°C. The autoradiographs were developed and positively hybridizing transformants were identified.

Bacterial stocks of purified hybridization positive clones were made and plasmid DNA was isolated. The sequence of the cDNA insert of each transformant was determined. In both instances, the isolated clones – AR2 and AR3 – were full-length as determined by a comparison with family members and the presence of a stop codon in the 5' UTR.

The nucleotide sequence of AR2 as presently determined is reported in SEQ ID NO:8. What applicants presently believe to be the proper reading frame and the predicted

amino acid sequence of the AR2 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:9. Clone AR2 was deposited on January 21, 1999 with the American Type Culture Collection under accession number ATCC 207060. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b).

The nucleotide sequence of AR3 as presently determined is reported in SEQ ID NO:13. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the AR3 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:14. Clone AR3 was deposited on January 21, 1999 with the American Type Culture Collection under accession number ATCC 207063. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b).

Apple Crisp Protein (APPLE) and Cocoa Crisp Protein (COCO)

A yeast signal sequence trap similar to that described in U.S. Patent No. 5,536,637 was used to identify secreted ESTs (sESTs) from a chicken embryonic limb bud library. An sEST was identified and used to clone a corresponding full-length chicken cDNA, GW159 (SEQ ID NO:15, SEQ ID NO:16).

Using the chicken GW159 full-length sequence to screen the public EST database, two partial human homolog sequences were identified. GenBank AA393537 is reported at SEQ ID NO:17 and GenBank AA247800 is reported at SEQ ID NO:18. Oligonucleotides were designed to these ESTs and full-length human clones were isolated.

The full-length cDNAs were isolated from a pool of dT-primed cDNA libraries constructed in the plasmid vector pED-dpc2. cDNA was made from human adult heart and human testes RNA purchased from CloneTech.

The probe sequence used to isolate Apple Crisp was derived from EST fragment AA393537 (SEQ ID NO:17). The sequence of the probe is provided as SEQ ID NO:19. The probe was antisense to the EST.

The probe sequence used to isolate Cocoa Crisp was derived from EST fragment AA247800 (SEQ ID NO:18). The sequence of the probe is provided as SEQ ID NO:22. The probe was also antisense to the EST.

The DNA probes were radioactively labeled with 32P and used to screen the human fetal heart dT-primed cDNA library, under high stringency hybridization/washing conditions. to identify clones containing sequences of the Apple Crisp and Cocoa Crisp genes.

One hundred thousand library transformants were plated at a density of approximately 5000 transformants per plate on separate sets of 20 plates each. Nitrocellulose replicas of the transformed colonies were hybridized to the 32P labeled DNA probes in standard hybridization buffer (6X SSC, 0.2% nonfat dried milk, 0.0008% sodium azide, 1% NP-40 and 0.05% sodium pyrophosphate) under high stringency conditions (65°C for 2 hours). After 2 hours hybridization, the radioactively labeled DNA probe containing hybridization solutions wre removed and the filters were washed under high stringency conditions (3X SSC, 0.05% sodium pyrophosphate, 21°C for 5 minutes standing; followed by 2.2X SSC, 0.05% sodium pyrophosphate, 21°C for 15 minutes shaking; followed by a second 2.2X SSC, 0.05% sodium pyrophosphate, 65°C for 1 minute shaking). The filters were wrapped in Saran wrap and exposed to x-ray film overnight to 3 days at –80°C. The autoradiographs were developed and positively hybridizing transformants of various signal intensities were identified.

These positive clones were picked, grown for 5 hours in selective medium and plated at low density (approximately 300 colonies per plate). Nitrocellulose replicas of the colonies were hybridized to the 32P labeled probes in standard hybridization buffer (6X SSC, 0.2% nonfat dried milk, 0.0008% sodium azide, 1% NP-40 and 0.05% sodium pyrophosphate) under high stringency conditions (65°C for 2 hours). After 2 hours hybridization, the radioactively labeled DNA probe containing hybridization solutions were removed and the filters were washed under high stringency conditions (3X SSC, 0.05% sodium pyrophosphate, 21°C for 5 minutes standing; followed by 2.2X SSC, 0.05% sodium pyrophosphate, 21°C for 15 minutes shaking; followed by a second 2.2X SSC, 0.05% sodium pyrophosphate, 65°C for 1 minute shaking). The filters were wrapped in

Saran wrap and exposed to x-ray film overnight to 3 days at –80°C. The autoradiographs were developed and positively hybridizing transformants were identified.

Bacterial stocks of purified hybridization positive clones were made and plasmid DNA was isolated. The sequence of the cDNA insert of each transformant was determined.

In both instances, the cDNA insert contained the sequence of the probe used in the hybridization and included a full-length open reading frame.

The nucleotide sequence of Apple Crisp (APPLE) as presently determined is reported in SEQ ID NO:20. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the Apple Crisp protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:21. Clone APPLE was deposited on January 21, 1999 with the American Type Culture Collection under accession number ATCC 207061. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b).

The nucleotide sequence of Cocoa Crisp (COCO) as presently determined is reported in SEQ ID NO:23. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the Cocoa Crisp protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:24. Clone COCO was deposited on January 21, 1999 with the American Type Culture Collection under accession number ATCC 207062. All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b).

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such

as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with ATCC) in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein may also be determinable from the amino acid sequence of the full-length form.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, *Trends Pharmacol. Sci.* 15(7): 250-254; Lavarosky *et al.*, 1997. *Biochem.*

Mol. Med. **62(1)**: 11-22; and Hampel, 1998. *Prog. Nucleic Acid Res. Mol. Biol.* **58**: 1-39; all of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the polynucleotide sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, *Bioessays* **14(9)**: 629-633; Zwaal *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* **90(16)**: 7431-7435; Clark *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* **91(2)**: 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour *et al.*, 1988, *Nature* **336**: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s).

Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. In such forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologues of the disclosed polynucleotides and proteins are also provided by the present invention. As used herein, a "species homologue" is a protein or polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide. Preferably, polynucleotide species homologues have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% identity) with the given polynucleotide, and protein species homologues have at least 30% sequence identity (more preferably, at least 45% identity; most preferably at least 60% identity) with the given protein, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides or the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Species homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species. Preferably, species homologues are those isolated from mammalian species. Most preferably, species homologues are those isolated from certain mammalian species such as, for example, *Pan troglodytes*, *Gorilla gorilla*, *Pongo pygmaeus*, *Hylobates concolor*, *Macaca mulatta*, *Papio papio*, *Papio hamadryas*, *Cercopithecus aethiops*, *Cebus capucinus*, *Aotus trivirgatus*, *Sanguinus oedipus*, *Microcebus murinus*, *Mus musculus*, *Rattus norvegicus*, *Cricetulus griseus*, *Felis catus*, *Mustela vison*, *Canis familiaris*, *Oryctolagus cuniculus*, *Bos taurus*, *Ovis aries*, *Sus scrofa*, and *Equus caballus*, for which

genetic maps have been created allowing the identification of syntenic relationships between the genomic organization of genes in one species and the genomic organization of the related genes in another species (O'Brien and Seuánez, 1988, *Ann. Rev. Genet.* **22**: 323-351; O'Brien *et al.*, 1993. *Nature Genetics* 3:103-112; Johansson *et al.*, 1995, *Genomics* **25**: 682-690; Lyons *et al.*, 1997, *Nature Genetics* **15**: 47-56; O'Brien *et al.*, 1997, *Trends in Genetics* **13(10)**: 393-399; Carver and Stubbs, 1997, *Genome Research* 7:1123-1137; all of which are incorporated by reference herein).

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotides which also encode proteins which are identical or have significantly similar sequences to those encoded by the disclosed polynucleotides. Preferably, allelic variants have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% identity) with the given polynucleotide, where sequence identity is determined by comparing the nucleotide sequences of the polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps. Allelic variants may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from individuals of the appropriate species.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

Stringency Condition	Polynucleotide Hybrid	Hybrid Length (bp)[1]	Hybridization Temperature and Buffer[2]	Wash Temperature and Buffer[2]
A	DNA:DNA	³ 50	65°C: 1xSSC -or- 42°C: 1xSSC, 50% formamide	65°C: 0.3xSSC
B	DNA:DNA	<50	T_B*: 1xSSC	T_B*: 1xSSC
C	DNA:RNA	³ 50	67°C: 1xSSC -or- 45°C: 1xSSC, 50% formamide	67°C: 0.3xSSC
D	DNA:RNA	<50	T_D*: 1xSSC	T_D*: 1xSSC
E	RNA:RNA	³ 50	70°C: 1xSSC -or- 50°C: 1xSSC, 50% formamide	70°C: 0.3xSSC
F	RNA:RNA	<50	T_F*: 1xSSC	T_F*: 1xSSC
G	DNA:DNA	³ 50	65°C: 4xSSC -or- 42°C: 4xSSC, 50% formamide	65°C: 1xSSC
H	DNA:DNA	<50	T_H*: 4xSSC	T_H*: 4xSSC
I	DNA:RNA	³ 50	67°C: 4xSSC -or- 45°C: 4xSSC, 50% formamide	67°C: 1xSSC
J	DNA:RNA	<50	T_J*: 4xSSC	T_J*: 4xSSC
K	RNA:RNA	³ 50	70°C: 4xSSC -or- 50°C: 4xSSC, 50% formamide	67°C: 1xSSC
L	RNA:RNA	<50	T_L*: 2xSSC	T_L*: 2xSSC
M	DNA:DNA	³ 50	50°C: 4xSSC -or- 40°C: 6xSSC, 50% formamide	50°C: 2xSSC
N	DNA:DNA	<50	T_N*: 6xSSC	T_N*: 6xSSC
O	DNA:RNA	³ 50	55°C: 4xSSC -or- 42°C: 6xSSC, 50% formamide	55°C: 2xSSC
P	DNA:RNA	<50	T_P*: 6xSSC	T_P*: 6xSSC
Q	RNA:RNA	³ 50	60°C: 4xSSC -or- 45°C: 6xSSC, 50% formamide	60°C: 2xSSC
R	RNA:RNA	<50	T_R*: 4xSSC	T_R*: 4xSSC

[1]: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

[2]: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

*T_B - T_R: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18

and 49 base pairs in length. $T_m(°C) = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\%G+C) - (600/N)$, where N is the number of bases in the hybrid, and $[Na^+]$ is the concentration of sodium ions in the hybridization buffer ($[Na^+]$ for 1xSSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin

(TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid

to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

USES AND BIOLOGICAL ACTIVITY

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

Angiogenic Activity of AR2 and AR3

As a member of the angiopoietin family of proteins, AR2 and AR3 promote angiogenesis. Thus, administration of AR2 or AR3 proteins may be used to promote the growth of vascular tissue where desirable, such as, for example, in wound healing (from trauma or as a result of surgical dissection) and transplantation.

Antagonists of AR2 and AR3 may also be used as inhibitors of angiogenesis. Thus, administration of such an antagonist may be used to inhibit the growth of vascular tissue where undesirable, such as, for example, in treating tumors. Antagonists of AR2 and AR3 would include antibodies (including without limitation, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies produced in transgenic non-human animals, and antigen-binding fragments thereof) which

bind such proteins. Such antibodies to AR2 and AR3 and other AR2- and AR3- binding species can be made in accordance with known methods.

Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such

as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

Nutritional Uses

Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

Cytokine and Cell Proliferation/Differentiation Activity

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G,

M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon g, Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti,

J.. Clark. S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include. without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections. or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia

gravis. graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also to be useful in the treatment of allergic reactions and conditions. such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions. in which immune suppression is desired (including, for example, organ transplantation). may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance. which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (*e.g.*, B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the immune cells without transmitting the corresponding costimulatory signal. Blocking B lymphocyte antigen function in this matter

prevents cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular blocking reagents in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins *in vivo* as described in Lenschow *et al.*, Science *257*:789-792 (1992) and Turka *et al.*, Proc. Natl. Acad. Sci USA, *89*:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function *in vivo* on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythmatosis in MRL/*lpr/lpr* mice or NZB hybrid mice, murine

autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the common cold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells *in vitro* with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the *in vitro* activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells *in vivo*.

In another application, up regulation or enhancement of antigen function (preferably B lymphocyte antigen function) may be useful in the induction of tumor immunity. Tumor cells (*e.g.*, sarcoma, melanoma, lymphoma, leukemia, neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. For example, tumor cells obtained from a patient can be transfected *ex vivo* with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on

the surface of the transfected cell. Alternatively, gene therapy techniques can be used to target a tumor cell for transfection *in vivo*.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessary costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I a chain protein and b_2 microglobulin protein or an MHC class II a chain protein and an MHC class II b chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol.

137:3494-3500. 1986; Bowmanet al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512. 1988; Bertagnolli et al., Cellular Immunology 133:327-341. 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991;

Zacharchuk. Journal of Immunology 145:4037-4045. 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

Hematopoiesis Regulating Activity

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e., in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc.., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation

employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

Activin/Inhibin Activity

A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin a family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin-b group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

Chemotactic/Chemokinetic Activity

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140. 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

Receptor/Ligand Activity

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology. Ed by J.E. Coligan, A.M. Kruisbeek, D.H.

Margulies, E.M. Shevach. W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989: Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

Anti-Inflammatory Activity

Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

Cadherin/Tumor Invasion Suppressor Activity

Cadherins are calcium-dependent adhesion molecules that appear to play major roles during development, particularly in defining specific cell types. Loss or alteration of normal cadherin expression can lead to changes in cell adhesion properties linked to tumor growth and metastasis. Cadherin malfunction is also implicated in other human diseases, such as pemphigus vulgaris and pemphigus foliaceus (auto-immune blistering skin diseases), Crohn's disease, and some developmental abnormalities.

The cadherin superfamily includes well over forty members, each with a distinct pattern of expression. All members of the superfamily have in common conserved extracellular repeats (cadherin domains), but structural differences are found in other parts of the molecule. The cadherin domains bind calcium to form their tertiary structure and thus calcium is required to mediate their adhesion. Only a few amino acids in the first cadherin domain provide the basis for homophilic adhesion; modification of this recognition site can change the specificity of a cadherin so that instead of recognizing only itself, the mutant molecule can now also bind to a different cadherin. In addition, some cadherins engage in heterophilic adhesion with other cadherins.

E-cadherin, one member of the cadherin superfamily, is expressed in epithelial cell types. Pathologically, if E-cadherin expression is lost in a tumor, the malignant cells become invasive and the cancer metastasizes. Transfection of cancer cell lines with polynucleotides expressing E-cadherin has reversed cancer-associated changes by returning altered cell shapes to normal, restoring cells' adhesiveness to each other and to their substrate, decreasing the cell growth rate, and drastically reducing anchorage-independent cell growth. Thus, reintroducing E-cadherin expression reverts carcinomas to a less advanced stage. It is likely that other cadherins have the same invasion suppressor role in carcinomas derived from other tissue types. Therefore, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be used to treat cancer. Introducing such proteins or polynucleotides into cancer cells can reduce or eliminate the cancerous changes observed in these cells by providing normal cadherin expression.

Cancer cells have also been shown to express cadherins of a different tissue type than their origin, thus allowing these cells to invade and metastasize in a different tissue in the body. Proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can be substituted in these cells for the inappropriately expressed cadherins, restoring normal cell adhesive properties and reducing or eliminating the tendency of the cells to metastasize.

Additionally, proteins of the present invention with cadherin activity, and polynucleotides of the present invention encoding such proteins, can used to generate antibodies recognizing and binding to cadherins. Such antibodies can be used to block the

adhesion of inappropriately expressed tumor-cell cadherins, preventing the cells from forming a tumor elsewhere. Such an anti-cadherin antibody can also be used as a marker for the grade, pathological type, and prognosis of a cancer, i.e. the more progressed the cancer, the less cadherin expression there will be, and this decrease in cadherin expression can be detected by the use of a cadherin-binding antibody.

Fragments of proteins of the present invention with cadherin activity, preferably a polypeptide comprising a decapeptide of the cadherin recognition site, and polynucleotides of the present invention encoding such protein fragments, can also be used to block cadherin function by binding to cadherins and preventing them from binding in ways that produce undesirable effects. Additionally, fragments of proteins of the present invention with cadherin activity, preferably truncated soluble cadherin fragments which have been found to be stable in the circulation of cancer patients, and polynucleotides encoding such protein fragments, can be used to disturb proper cell-cell adhesion.

Assays for cadherin adhesive and invasive suppressor activity include, without limitation, those described in: Hortsch et al. J Biol Chem 270 (32): 18809-18817, 1995; Miyaki et al. Oncogene 11: 2547-2552, 1995; Ozawa et al. Cell 63: 1033-1038, 1990.

Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth.

Other Activities

A protein of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting

(suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF,

thrombopoietin. stem cell factor. and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention. or to minimize side effects. Conversely. protein of the present invention may be included in formulations of the particular cytokine. lymphokine. other hematopoietic factor. thrombolytic or anti-thrombotic factor. or anti-inflammatory agent to minimize side effects of the cytokine. lymphokine. other hematopoietic factor. thrombolytic or anti-thrombotic factor. or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunolgobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined. in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals. or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include. without limitation,

monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.

When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses

of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 mg to about 100 mg (preferably about 0.1ng to about 10 mg, more preferably about 0.1 mg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When

administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorbtion of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF-a and TGF-b), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.

Patent and literature references cited herein are incorporated by reference as if fully set forth.

What is claimed is:

1. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:8 from nucleotide 433 to nucleotide 1905;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR2 deposited under accession number ATCC 207060;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR2 deposited under accession number ATCC 207060;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:9;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:9 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

2. The polynucleotide of claim 1 wherein said polynucleotide is operably linked to at least one expression control sequence.

3. A host cell transformed with the polynucleotide of claim 2.

4. The host cell of claim 3, wherein said cell is a mammalian cell.

5. A process for producing a protein encoded by the polynucleotide of claim 2, which process comprises:

(a) growing a culture of the host cell of claim 3 in a suitable culture medium; and

(b) purifying said protein from the culture.

6. A protein produced according to the process of claim 5.

7. The protein of claim 6 comprising a mature protein.

8. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:9;

(b) fragments of the amino acid sequence of SEQ ID NO:9; and

(c) the amino acid sequence encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060;

the protein being substantially free from other mammalian proteins.

9. The protein of claim 8, wherein said protein comprises the amino acid sequence of SEQ ID NO:9.

10. The protein of claim 8, wherein said protein comprises the amino acid sequence encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060.

11. A composition comprising the protein of claim 8 and a pharmaceutically acceptable carrier.

12. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 11.

13. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:8.

14. The polynucleotide of claim 1, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:8.

15. The polynucleotide of claim 1, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:8 from nucleotide 433 to nucleotide 1905.

16. The polynucleotide of claim 1, wherein the polynucleotide comprises the nucleotide sequence of the full-length protein coding sequence of clone AR2 deposited under accession number ATCC 207060.

17. The polynucleotide of claim 1, wherein the polynucleotide encodes the full-length protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060.

18. The polynucleotide of claim 1, wherein the polynucleotide comprises the nucleotide sequence of the mature protein coding sequence of AR2 deposited under accession number ATCC 207060.

19. The polynucleotide of claim 1, wherein the polynucleotide encodes the mature protein encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060.

20. The polynucleotide of claim 1, wherein the polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:9.

21. The polynucleotide of claim 1, wherein the polynucleotide encodes a protein comprising a fragment of the amino acid sequence of SEQ ID NO:9 having biological activity.

22. The protein of claim 8, wherein the protein comprises a fragment of the amino acid sequence of SEQ ID NO:9 having biological activity.

23. The protein of claim 8, wherein the protein comprises the amino acid sequence encoded by the cDNA insert of clone AR2 deposited under accession number ATCC 207060.

24. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 from nucleotide 358 to nucleotide 1836;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone AR3 deposited under accession number ATCC 207063;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone AR3 deposited under accession number ATCC 207063;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:14;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

25. The polynucleotide of claim 24 wherein said polynucleotide is operably linked to at least one expression control sequence.

26. A host cell transformed with the polynucleotide of claim 25.

27. The host cell of claim 26, wherein said cell is a mammalian cell.

28. A process for producing a protein encoded by the polynucleotide of claim 25, which process comprises:

(a) growing a culture of the host cell of claim 6 in a suitable culture medium; and

(b) purifying said protein from the culture.

29. A protein produced according to the process of claim 28.

30. The protein of claim 29 comprising a mature protein.

31. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:14;

(b) fragments of the amino acid sequence of SEQ ID NO:14; and

(c) the amino acid sequence encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063;

the protein being substantially free from other mammalian proteins.

32. The protein of claim 31, wherein said protein comprises the amino acid sequence of SEQ ID NO:14.

33. The protein of claim 31, wherein said protein comprises the amino acid sequence encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063.

34. A composition comprising the protein of claim 31 and a pharmaceutically acceptable carrier.

35. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 34.

36. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:13.

37. The polynucleotide of claim 24, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:13.

38. The polynucleotide of claim 24, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:13 from nucleotide 358 to nucleotide 1836.

39. The polynucleotide of claim 24, wherein the polynucleotide comprises the nucleotide sequence of the full-length protein coding sequence of clone AR3 deposited under accession number ATCC 207063.

40. The polynucleotide of claim 24, wherein the polynucleotide encodes the full-length protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063.

41. The polynucleotide of claim 24, wherein the polynucleotide comprises the nucleotide sequence of the mature protein coding sequence of AR3 deposited under accession number ATCC 207063.

42. The polynucleotide of claim 24, wherein the polynucleotide encodes the mature protein encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063.

43. The polynucleotide of claim 24, wherein the polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:14.

44. The polynucleotide of claim 24, wherein the polynucleotide encodes a protein comprising a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity.

45. The protein of claim 31, wherein the protein comprises a fragment of the amino acid sequence of SEQ ID NO:14 having biological activity.

46. The protein of claim 31, wherein the protein comprises the amino acid sequence encoded by the cDNA insert of clone AR3 deposited under accession number ATCC 207063.

47. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:20;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:20 from nucleotide 50 to nucleotide 775;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone APPLE deposited under accession number ATCC 207061;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone APPLE deposited under accession number ATCC 207061;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:21;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:21 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

48. The polynucleotide of claim 47 wherein said polynucleotide is operably linked to at least one expression control sequence.

49. A host cell transformed with the polynucleotide of claim 48.

50. The host cell of claim 49, wherein said cell is a mammalian cell.

51. A process for producing a protein encoded by the polynucleotide of claim 48, which process comprises:

(a) growing a culture of the host cell of claim 49 in a suitable culture medium; and

(b) purifying said protein from the culture.

52. A protein produced according to the process of claim 51.

53. The protein of claim 52 comprising a mature protein.

54. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:21;

(b) fragments of the amino acid sequence of SEQ ID NO:21; and

(c) the amino acid sequence encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061;

the protein being substantially free from other mammalian proteins.

55. The protein of claim 54, wherein said protein comprises the amino acid sequence of SEQ ID NO:21.

56. The protein of claim 54, wherein said protein comprises the amino acid sequence encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061.

57. A composition comprising the protein of claim 54 and a pharmaceutically acceptable carrier.

58. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 57.

59 An isolated gene corresponding to the cDNA sequence of SEQ ID NO:20.

60. The polynucleotide of claim 47, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:20.

61. The polynucleotide of claim 47, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:20 from nucleotide 50 to nucleotide 775.

62. The polynucleotide of claim 47, wherein the polynucleotide comprises the nucleotide sequence of the full-length protein coding sequence of clone APPLE deposited under accession number ATCC 207061.

63. The polynucleotide of claim 47, wherein the polynucleotide encodes the full-length protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061.

64. The polynucleotide of claim 47, wherein the polynucleotide comprises the nucleotide sequence of the mature protein coding sequence of APPLE deposited under accession number ATCC 207061.

65. The polynucleotide of claim 47, wherein the polynucleotide encodes the mature protein encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061.

66. The polynucleotide of claim 47, wherein the polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:21.

67. The polynucleotide of claim 47, wherein the polynucleotide encodes a protein comprising a fragment of the amino acid sequence of SEQ ID NO:21 having biological activity.

68. The protein of claim 54, wherein the protein comprises a fragment of the amino acid sequence of SEQ ID NO:21 having biological activity.

69. The protein of claim 54, wherein the protein comprises the amino acid sequence encoded by the cDNA insert of clone APPLE deposited under accession number ATCC 207061.

70. An isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:23;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:23 from nucleotide 65 to nucleotide 1564;

(c) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone COCO deposited under accession number ATCC 207062;

(d) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062;

(e) a polynucleotide comprising the nucleotide sequence of the mature protein coding sequence of clone COCO deposited under accession number ATCC 207062;

(f) a polynucleotide encoding the mature protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062;

(g) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:24;

(h) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:24 having biological activity;

(i) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(j) a polynucleotide which encodes a species homologue of the protein of (f) or (g) above ; and

(k) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i).

71. The polynucleotide of claim 70 wherein said polynucleotide is operably linked to at least one expression control sequence.

72. A host cell transformed with the polynucleotide of claim 71.

73. The host cell of claim 72, wherein said cell is a mammalian cell.

74. A process for producing a protein encoded by the polynucleotide of claim 71, which process comprises:

(a) growing a culture of the host cell of claim 72 in a suitable culture medium; and

(b) purifying said protein from the culture.

75. A protein produced according to the process of claim 74.

76. The protein of claim 75 comprising a mature protein.

77. A protein comprising an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:24;

(b) fragments of the amino acid sequence of SEQ ID NO:24; and

(c) the amino acid sequence encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062;

the protein being substantially free from other mammalian proteins.

78. The protein of claim 77, wherein said protein comprises the amino acid sequence of SEQ ID NO:24.

79. The protein of claim 77, wherein said protein comprises the amino acid sequence encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062.

80. A composition comprising the protein of claim 77 and a pharmaceutically acceptable carrier.

81. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 80.

82. An isolated gene corresponding to the cDNA sequence of SEQ ID NO:23.

83. The polynucleotide of claim 70, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:23.

84. The polynucleotide of claim 70, wherein the polynucleotide comprises the nucleotide sequence of SEQ ID NO:23 from nucleotide 65 to nucleotide 1564.

85. The polynucleotide of claim 70, wherein the polynucleotide comprises the nucleotide sequence of the full-length protein coding sequence of clone COCO deposited under accession number ATCC 207062.

86. The polynucleotide of claim 70, wherein the polynucleotide encodes the full-length protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062.

87. The polynucleotide of claim 70, wherein the polynucleotide comprises the nucleotide sequence of the mature protein coding sequence of COCO deposited under accession number ATCC 207062.

88. The polynucleotide of claim 70, wherein the polynucleotide encodes the mature protein encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062.

89. The polynucleotide of claim 70, wherein the polynucleotide encodes a protein comprising the amino acid sequence of SEQ ID NO:24.

90. The polynucleotide of claim 70, wherein the polynucleotide encodes a protein comprising a fragment of the amino acid sequence of SEQ ID NO:24 having biological activity.

91. The protein of claim 77, wherein the protein comprises a fragment of the amino acid sequence of SEQ ID NO:24 having biological activity.

92. The protein of claim 77, wherein the protein comprises the amino acid sequence encoded by the cDNA insert of clone COCO deposited under accession number ATCC 207062.

93. An antibody which specifically reacts with the protein of claim 8.

94. An antibody which specifically reacts with the protein of claim 31.

95. An antibody which specifically reacts with the protein of claim 54.

96. An antibody which specifically reacts with the protein of claim 77.

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<210> 2
<211> 491
<212> PRT
<213> Gallus gallus

<400> 2

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Met Lys Ile Leu Lys Trp Thr Leu Gly Met Leu Leu Phe Leu Leu Leu
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Ser Ile Gly Arg Cys Thr Glu Pro Ser Thr Leu Asn Lys Thr Ser Gln
            20                  25                  30

Arg Arg His Pro Arg Ser Thr Asp Gly Gly Glu Glu Gly Arg Lys Cys
        35                  40                  45

Gly Tyr Thr Phe Leu Val Pro Glu Gln Lys Ile Thr Gly Pro Ile Cys
    50                  55                  60

Val Asn Asn Glu Pro Gly Thr Gly Asn Arg Lys Asp Glu Val Thr Arg
65                  70                  75                  80

Met Asp Ile Glu Asn Leu Lys Asp Val Leu Ser Lys Gln Lys Arg Glu
                85                  90                  95

Ile Asp Ile Leu Gln Leu Val Val Asp Val Asp Gly Asn Ile Val Asn
            100                 105                 110

Glu Val Lys Leu Leu Arg Lys Glu Ser Arg Asn Met Asn Ser Arg Val
        115                 120                 125

Thr Gln Leu Tyr Met Gln Leu Leu His Glu Ile Ile Arg Lys Arg Asp
    130                 135                 140

Asn Ser Leu Glu Leu Ser Gln Leu Glu Asn Lys Val Leu Asn Val Thr
145                 150                 155                 160

Thr Glu Met Leu Lys Met Ala Thr Lys Tyr Lys Glu Leu Glu Val Lys
                165                 170                 175

Tyr Ala Ala Leu Thr Asp Leu Val Asn Asn Gln Ser Val Ile Ile Ser
            180                 185                 190

Leu Leu Glu Glu Gln Cys Leu Arg Ile Phe Ser Arg Gln Asp Thr His
        195                 200                 205

Gly Ser Pro Pro Leu Val Gln Val Val Pro Gln His Ile Pro Asn Ser
    210                 215                 220

Gln Pro Tyr Thr Pro Val Leu Leu Gly Gly Asn Glu Ile Gln Arg Asp
225                 230                 235                 240

Pro Gly Tyr Pro Arg Asp Arg Asp Val Arg Pro Pro Pro Asp Pro Ala
                245                 250                 255

Thr Ser Pro Thr Lys Ser Pro Phe Arg Val Pro Pro Leu Ala Leu Ile
            260                 265                 270

Asn Glu Gly Pro Phe Lys Asp Cys Gln His Ala Lys Asp Ala Gly Tyr
        275                 280                 285

Ser Asn Ser Gly Ile Tyr Met Ile Lys Ala Glu Asn Ser Asn Glu Pro
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290 295 300

Met Gln Leu Trp Cys Glu Asn Ser Leu Asp Pro Gly Gly Trp Ala Val
305 310 315 320

Ile Gln Lys Arg Thr Asp Gly Ser Val Asn Phe Phe Arg Asn Trp Asp
325 330 335

Ser Tyr Lys Lys Gly Phe Gly Asn Ile Asp Gly Glu Tyr Trp Leu Gly
340 345 350

Leu Glu Asn Ile Tyr Met Leu Thr Asn Gln Asp Asn Tyr Arg Leu Leu
355 360 365

Ile Glu Leu Glu Asp Trp Ser Asn Lys Lys Val Tyr Ala Glu Tyr Ser
370 375 380

Ser Phe Arg Leu Glu Pro Glu Asn Glu Phe Tyr Arg Leu Arg Leu Gly
385 390 395 400

Thr Tyr Gln Gly Asn Ala Gly Asp Ser Met Ile Trp His Asn Gly Lys
405 410 415

Gln Phe Thr Thr Leu Asp Arg Asp Arg Asp Met Tyr Ser Gly Asn Cys
420 425 430

Ala His Phe His Lys Gly Gly Trp Trp Tyr Asn Ala Cys Ala His Ser
435 440 445

Asn Leu Asn Gly Val Trp Tyr Arg Gly Gly His Tyr Arg Ser Lys Tyr
450 455 460

Gln Asp Gly Ile Phe Trp Ala Glu Tyr Arg Gly Gly Ser Tyr Ser Leu
465 470 475 480

Lys Ala Val Gln Met Met Ile Arg Pro Ile Asp
485 490

<210> 3
<211> 323
<212> DNA
<213> Homo sapiens

<400> 3
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gattgaatta gaagactgga gtgataaaaa agtctatgca gaatacagca gctttcgtct 240
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ttctatgatg tggcataatg gta 323

<210> 4
<211> 465
<212> DNA
<213> Homo sapiens

<400> 4
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ccgggggctg gaccgtcatc cagagacgcc tggatggctc tgttaacttc ttcaggaact 180
gggagacgta caagcaaggg tttgggaaca ttgacggcga atactggctg ggcctggaga 240
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ccggccgcaa agtctttgca gaatacgcca gtttccgcct ggaacctgag agcgagtatt 360
ataagctgcg gctggggcgc taccatggca atgcgggtga ctcctttaca tggcacaacg 420
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<210> 5
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
<221> unsure
<222> (1)

<220>
<221> misc_feature
<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 5
nnacccccag ggtccaaact gttttcaca 29

<210> 6
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
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<222> (1)

<220>
<221> misc_feature
<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 6
nnctgaagaa gttgacagag ccgtctgtt 29

<210> 7
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
<221> unsure
<222> (1)

<220>
<221> misc_feature
<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 7
nngaagcgta tttcacctct agttccctg 29

<210> 8
<211> 2430
<212> DNA
<213> Homo sapiens

<400> 8
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ccacagacca gaacttccac tatactactt aaaattacat aggtggcttg tcaaattcaa 120
ttgattagta ttgtaaaagg aaaaagaagt tccttcttac agcttggatt caacggtcca 180
aaacaaaaat gcagctgcca ttaaagtcac agatgaacaa acttctacac tgatttttaa 240
aatcaagaat aagggcagca agtttctgga ttcactgaat caacagacac aaaaagctgg 300
caatatagca actatgaaga gaaaagctac taataaaatt aacccaacgc atagaagact 360
ttttttttct ttctaaaaac aactaagtaa agacttaaat ttaaacacat cattttacaa 420
cctcatttca aaatgaagac ttttacctgg accctaggtg tgctattctt cctactagtg 480
gacactggac attgcagagg tggacaattc aaaattaaaa aaataaacca gagaagatac 540
cctcgtgcca cagatggtaa agaggaagca aagaaatgtg catacacatt cctggtacct 600
gaacaaagaa taacagggcc aatctgtgtc aacaccaagg ggcaagatgc aagtaccatt 660
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aattagggag aaacctctag ttttgccaat agaaaatgct tcttccattg aataaaagtt 2400
atttcaaatt gaaaaaaaaa aaaaaaaaaa 2430

<210> 9
<211> 491
<212> PRT
<213> Homo sapiens

<400> 9
Met Lys Thr Phe Thr Trp Thr Leu Gly Val Leu Phe Phe Leu Leu Val
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Asp Thr Gly His Cys Arg Gly Gly Gln Phe Lys Ile Lys Lys Ile Asn
20 25 30

Gln Arg Arg Tyr Pro Arg Ala Thr Asp Gly Lys Glu Glu Ala Lys Lys
35 40 45

Cys Ala Tyr Thr Phe Leu Val Pro Glu Gln Arg Ile Thr Gly Pro Ile
50 55 60

Cys Val Asn Thr Lys Gly Gln Asp Ala Ser Thr Ile Lys Asp Met Ile
65 70 75 80

Thr Arg Met Asp Leu Glu Asn Leu Lys Asp Val Leu Ser Arg Gln Lys
85 90 95

Arg Glu Ile Asp Val Leu Gln Leu Val Val Asp Val Asp Gly Asn Ile
100 105 110

Val Asn Glu Val Lys Leu Leu Arg Lys Glu Ser Arg Asn Met Asn Ser
115 120 125

Arg Val Thr Gln Leu Tyr Met Gln Leu Leu His Glu Ile Ile Arg Lys
130 135 140

Arg Asp Asn Ser Leu Glu Leu Ser Gln Leu Glu Asn Lys Ile Leu Asn
145 150 155 160

Val Thr Thr Glu Met Leu Lys Met Ala Thr Arg Tyr Arg Glu Leu Glu
165 170 175

Val Lys Tyr Ala Ser Leu Thr Asp Leu Val Asn Asn Gln Ser Val Met
180 185 190

Ile Thr Leu Leu Glu Glu Gln Cys Leu Arg Ile Phe Ser Arg Gln Asp
195 200 205

Thr His Val Ser Pro Pro Leu Val Gln Val Val Pro Gln His Ile Pro
210 215 220

Asn Ser Gln Gln Tyr Thr Pro Gly Leu Leu Gly Gly Asn Glu Ile Gln
225 230 235 240

Arg Asp Pro Gly Tyr Pro Arg Asp Leu Met Pro Pro Pro Asp Leu Ala
245 250 255

Thr Ser Pro Thr Lys Ser Pro Phe Lys Ile Pro Pro Val Thr Phe Ile
260 265 270

Asn Glu Gly Pro Phe Lys Asp Cys Gln Gln Ala Lys Glu Ala Gly His
275 280 285

Ser Val Ser Gly Ile Tyr Met Ile Lys Pro Glu Asn Ser Asn Gly Pro
290 295 300

Met Gln Leu Trp Cys Glu Asn Ser Leu Asp Pro Gly Gly Trp Thr Val
305 310 315 320

Ile Gln Lys Arg Thr Asp Gly Ser Val Asn Phe Phe Arg Asn Trp Glu
325 330 335

Asn Tyr Lys Lys Gly Phe Gly Asn Ile Asp Gly Glu Tyr Trp Leu Gly
340 345 350

Leu Glu Asn Ile Tyr Met Leu Ser Asn Gln Asp Asn Tyr Lys Leu Leu
355 360 365

Ile Glu Leu Glu Asp Trp Ser Asp Lys Lys Val Tyr Ala Glu Tyr Ser
370 375 380

Ser Phe Arg Leu Glu Pro Glu Ser Glu Phe Tyr Arg Leu Arg Leu Gly
385 390 395 400

Thr Tyr Gln Gly Asn Ala Gly Asp Ser Met Met Trp His Asn Gly Lys
405 410 415

Gln Phe Thr Thr Leu Asp Arg Asp Lys Asp Met Tyr Ala Gly Asn Cys
420 425 430

Ala His Phe His Lys Gly Gly Trp Trp Tyr Asn Ala Cys Ala His Ser
435 440 445

Asn Leu Asn Gly Val Trp Tyr Arg Gly Gly His Tyr Arg Ser Lys His
450 455 460

Gln Asp Gly Ile Phe Trp Ala Glu Tyr Arg Gly Gly Ser Tyr Ser Leu
465 470 475 480

Arg Ala Val Gln Met Met Ile Lys Pro Ile Asp
485 490

<210> 10
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
<221> unsure

<222> (1)

<220>
<221> misc_feature
<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 10
nngaactgct tgccgttgtg ccatgtaaa 29

<210> 11
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
<221> unsure
<222> (1)

<220>
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<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 11
nnttcaccag gtagatggag ctggtgtcg 29

<210> 12
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide

<220>
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<222> (1)

<220>
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<222> (2)
<223> biotinylated phosphoaramidite residue

<400> 12
nngatctggt tgatgatgcg gttgtaggt 29

<210> 13
<211> 2154
<212> DNA
<213> Homo sapiens

<400> 13
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cggctgacgc tactgtgagg gaaagaaggt tgtgagcagc cccgcaggac ccctggccag 120
ccctggcccc agcctctgcc ggagccctct gtggaggcag agccagtgga gcccagtgag 180
gcagggctgc ttggcagcca ccggcctgca actcaggaac ccctccagag gccatggaca 240
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aaccgagtgc ataagcagga gctagagctg ctcaacaatg agctgctcaa gcagaagcgg 660
cagatcgaga cgctgcagca gctggtggag gtggacggcg gcattgtgag cgaggtgaag 720
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<210> 14
<211> 493
<212> PRT
<213> Homo sapiens

<400> 14
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Met Gly Ala Val Ala Gly Gln Glu Asp Gly Phe Glu Gly Thr Glu Glu
20 25 30

Gly Ser Pro Arg Glu Phe Ile Tyr Leu Asn Arg Tyr Lys Arg Ala Gly
35 40 45

Glu Ser Gln Asp Lys Cys Thr Tyr Thr Phe Ile Val Pro Gln Gln Arg
50 55 60

Val Thr Gly Ala Ile Cys Val Asn Ser Lys Glu Pro Glu Val Leu Leu
65 70 75 80

Glu Asn Arg Val His Lys Gln Glu Leu Glu Leu Leu Asn Asn Glu Leu
85 90 95

Leu Lys Gln Lys Arg Gln Ile Glu Thr Leu Gln Gln Leu Val Glu Val
100 105 110

Asp Gly Gly Ile Val Ser Glu Val Lys Leu Leu Arg Lys Glu Ser Arg
115 120 125

Asn Met Asn Ser Arg Val Thr Gln Leu Tyr Met Gln Leu Leu His Glu
130 135 140

Ile Ile Arg Lys Arg Asp Asn Ala Leu Glu Leu Ser Gln Leu Glu Asn
145 150 155 160

Arg Ile Leu Asn Gln Thr Ala Asp Met Leu Gln Leu Ala Ser Lys Tyr
165 170 175

Lys Asp Leu Glu His Lys Tyr Gln His Leu Ala Thr Leu Ala His Asn
180 185 190

Gln Ser Glu Ile Ile Ala Gln Leu Glu Glu His Cys Gln Arg Val Pro
195 200 205

Ser Ala Arg Pro Val Pro Gln Pro Pro Pro Ala Ala Pro Pro Arg Val
210 215 220

Tyr Gln Pro Pro Thr Tyr Asn Arg Ile Ile Asn Gln Ile Ser Thr Asn
225 230 235 240

Glu Ile Gln Ser Asp Gln Asn Leu Lys Val Leu Pro Pro Pro Leu Pro
245 250 255

Thr Met Pro Thr Leu Thr Ser Leu Pro Ser Ser Thr Asp Lys Pro Ser
260 265 270

Gly Pro Trp Arg Asp Cys Leu Gln Ala Leu Glu Asp Gly His Asp Thr
275 280 285

Ser Ser Ile Tyr Leu Val Lys Pro Glu Asn Thr Asn Arg Leu Met Gln
290 295 300

Val Trp Cys Asp Gln Arg His Asp Pro Gly Gly Trp Thr Val Ile Gln
305 310 315 320

Arg Arg Leu Asp Gly Ser Val Asn Phe Phe Arg Asn Trp Glu Thr Tyr
325 330 335

Lys Gln Gly Phe Gly Asn Ile Asp Gly Glu Tyr Trp Leu Gly Leu Glu
340 345 350

Asn Ile Tyr Trp Leu Thr Asn Gln Gly Asn Tyr Lys Leu Leu Val Thr
355 360 365

Met Glu Asp Trp Ser Gly Arg Lys Val Phe Ala Glu Tyr Ala Ser Phe
370 375 380

Arg Leu Glu Pro Glu Ser Glu Tyr Tyr Lys Leu Arg Leu Gly Arg Tyr
385 390 395 400

His Gly Asn Ala Gly Asp Ser Phe Thr Trp His Asn Gly Lys Gln Phe
405 410 415

Thr Thr Leu Asp Arg Asp His Asp Val Tyr Thr Gly Asn Cys Ala His
420 425 430

Tyr Gln Lys Gly Gly Trp Trp Tyr Asn Ala Cys Ala His Ser Asn Leu
435 440 445

Asn Gly Val Trp Tyr Arg Gly Gly His Tyr Arg Ser Arg Tyr Gln Asp
450 455 460

Gly Val Tyr Trp Ala Glu Phe Arg Gly Gly Ser Tyr Ser Leu Lys Lys
465 470 475 480

Val Val Met Met Ile Arg Pro Asn Pro Asn Thr Phe His
485 490

<210> 15
<211> 1394
<212> DNA
<213> Gallus gallus

<400> 15
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agagacactc cttcacaaag gttcatatca tcacatagga gagtgttata tatactggga 180
gaacaataga ctgtattcat tgtggtatta tccaaagatc tgtttttctac tgccctgact 240
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tgcaatcagt tgtgttttct tattttccat tctctgtgaa acaagtgcat tagtgttacc 360
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aaaaaaaaaa aaaa 1394

<210> 16
<211> 258
<212> PRT
<213> Gallus gallus

<400> 16
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Cys Glu Thr Ser Ala Leu Val Leu Pro Asn Ser Thr Asp Leu Leu Leu
20 25 30

Ser Asn Asn Asn Phe Thr Asp Ile Glu Thr Ala Leu Ala Ala His Leu
35 40 45

Asp Ser Ala Lys Ile Pro Lys Ala Arg Arg Lys Arg Tyr Ile Ser Gln
50 55 60

Asn Asp Met Ile Ala Ile Leu Asp Tyr His Asn Gln Val Arg Gly Lys
65 70 75 80

Val Phe Pro Pro Ala Ser Asn Met Glu Tyr Met Val Trp Asp Glu Thr
85 90 95

Leu Ala Lys Ser Ala Glu Ala Trp Ala Ala Thr Cys Ile Trp Asp His
100 105 110

Gly Pro Ser Tyr Leu Leu Arg Phe Leu Gly Gln Asn Leu Ser Val Arg
115 120 125

Thr Gly Arg Tyr Arg Ser Ile Leu Gln Leu Val Lys Pro Trp Tyr Asp
130 135 140

Glu Val Lys Asp Tyr Ala Phe Pro Tyr Pro Gln Asp Cys Asn Pro Arg
145 150 155 160

Cys Pro Met Arg Cys Tyr Gly Pro Met Cys Thr His Tyr Thr Gln Met
165 170 175

Val Trp Ala Thr Ser Asn Arg Ile Gly Cys Ala Ile His Thr Cys Gln
180 185 190

Asn Met Asn Val Trp Gly Ser Val Trp Arg Arg Ala Val Tyr Leu Val
195 200 205

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210 215 220

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Ile Leu Asp Leu His Asn Lys Leu Arg Ser Gln Val Tyr Pro Thr Ala
65 70 75 80

Ser Asn Met Glu Tyr Met Thr Trp Asp Val Glu Leu Glu Arg Ser Ala
85 90 95

Glu Ser Trp Ala Glu Ser Cys Leu Trp Glu His Gly Pro Ala Ser Leu
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Pro Pro Thr Phe His Val Gln Ser Trp Tyr Asp Glu Val Lys Asp Phe

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Ser Gly Pro Val Cys Thr His Tyr Thr Gln Val Val Trp Ala Thr Ser
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Cys Arg Ala Ala Ile His Tyr Gly Ile Ile Asp Asn Asp Gly Gly Trp
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Val Asp Ile Thr Arg Gln Gly Arg Lys His Tyr Phe Ile Lys Ser Asn
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Arg Asn Gly Ile Gln Thr Ile Gly Lys Tyr Gln Ser Ala Asn Ser Phe
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Phe Ala Val Val
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/08832

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350, 351, 387.1, 387.9; 514/2, 8, 12, 885; 435/69.1, 69.5, 71.1, 71.2, 471, 320.1, 252.3, 254.11, 325; 428/85.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, CAS ONLINE, MEDLINE, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- A	WO 92/05256 A1 (GENETICS INSTITUTE, INC.) 02 April 1992 (02/04/92), see entire document, especially pages 17-21.	1-7, 21-22, 24-30, 47-53, 67-68, 70-76 --- 8-20, 23, 31-46, 54-66, 69, 77-96

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 24 JULY 2000	Date of mailing of the international search report 09 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/08832

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (7):

C07K 14/47, 14/515, 14/52, 16/22, 16/24; A61K 38/17, 38/18, 38/19; C12N 5/10, 15/12, 15/19

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

530/350, 351, 387.1, 387.9; 514/2, 8, 12, 885; 435/69.1, 69.5, 71.1, 71.2, 471, 320.1, 252.3, 254.11, 325; 428/85.1